Universidade de Lisboa

Faculdade de Medicina Dentária

Effect of Chlorhexidine Loading on the Flexural Strength of Acrylic Reline Resins After Thermal Ageing

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Dissertação

Mestrado Integrado em Medicina Dentária

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Dissertação orientada pela Professora Doutora Cristina Bettencourt Neves e coorientada pelo Professor Doutor Jaime Portugal

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Table of Contents

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Resumo

A reabsorção do rebordo alveolar associada à perda dentária é um fenómeno fisiológico crónico e progressivo de remodelação óssea. Por essa razão, indivíduos que possuam próteses dentárias removíveis vão sendo submetidos a uma progressiva perda de estabilidade e retenção da prótese e, consequentemente, um crescente desconforto. Como forma de corrigir esta desadaptação, recorrem-se a rebasamentos, utilizando, para tal, resinas acrílicas auto-polimerizáveis cuja polimerização poderá ser classificada como direta ou indireta, consoante o ambiente onde ocorre, na cavidade oral ou em laboratório, respetivamente.

A estomatite protética é uma patologia inflamatória que afeta, com frequência, indivíduos reabilitados com próteses dentárias removíveis. Esta patologia demonstra uma etiologia multifatorial, necessitando do sinergismo de um conjunto de fatores, tais como, o trauma associado a uma prótese instável e desadaptada, a idade elevada do indivíduo, uma fraca higienização oral e da prótese, xerostomia, ausência de descanso noturno da prótese e a presença de uma doença crónica ou comprometimento imunitário. Para além destes fatores etiológicos, é muito relevante a ação da espécie *Candida*, especialmente, o patógeno *Candida albicans*, que facilmente adere e prolifera nos tecidos da cavidade oral e nas resinas acrílicas, gerando biofilmes heterogéneos e complexos.

O tratamento da estomatite protética envolve, por um lado, a adaptação da prótese removível, a confeção de uma nova prótese dentária ou a limpeza da prótese removível pré-existente e, por outro, a terapia farmacológica, sendo que os fármacos antifúngicos disponibilizados poderão ser administrados de forma tópica ou sistémica. A terapia antifúngica revela algumas desvantagens, nomeadamente, a necessidade da cooperação do paciente e o rápido desvanecimento do fármaco do local alvo. Para além destas desvantagens, quando administrados de forma sistémica, os fármacos antifúngicos alcançam o local de infeção em concentrações muito baixas, acrescendo daí um risco de existirem reações secundárias adversas.

A baixa eficácia do tratamento convencional da estomatite protética impulsionou o surgimento de novas medidas. A incorporação dos agentes terapêuticos anteriormente referidos em materiais de rebasamento ou da base da prótese tem sido uma alternativa estudada. O propósito da criação destes dispositivos transportadores de fármacos é o de

libertar no local de infeção, em doses adequadas e contínuas, o agente terapêutico, sem necessitar do envolvimento do paciente.

A Clorexidina (CHX) é um fármaco antisético com ação antibacteriana, antifúngica e anti-biofilme de elevada substantividade. O facto de ser capaz de suprimir a adesão da *Candida albicans* às células da mucosa oral e às superfícies das resinas torna este fármaco um bom candidato para o tratamento da estomatite protética. Adicionalmente, estudos microbiológicos comprovaram uma eficácia superior comparativamente a outros agentes antifúngicos como o fluconazol e a nistatina.

Este fármaco, quando incorporado em resinas acrílicas apresenta uma taxa de eluição durante um período de vinte e oito dias, sendo que esta libertação é mais elevada nos primeiros dois a sete dias após o que se torna mais baixa e estável até ao fim do referido intervalo de tempo.

Alguns autores investigaram também, a ação que a incorporação de CHX tem em diversas propriedades mecânicas de resinas de rebasamento. Destes estudos foi possível concluir que incorporar CHX no valor de 10% da massa do pó das resinas de rebasamento Kooliner e Probase Cold tem efeitos nocivos na resistência à flexão destas, tal como na concentração de 7,5% na resina Probase Cold.

Tendo como referência as proporções de CHX previamente estudadas, o objetivo deste estudo foi avaliar a influência da incorporação que diferentes concentrações de CHX têm sobre a resistência à flexão das resinas Kooliner, Ufi Gel Hard e Probase Cold, após estas terem sido sujeitas a um processo de envelhecimento térmico que simula o ambiente da cavidade oral.

Foram preparados espécimes (64x10x3,3 mm), de cada uma das resinas, recorrendo a moldes retangulares de aço inoxidável. Para todas as resinas foi executado um grupo controlo com uma concentração de CHX de 0% da massa do pó do material, sendo que para a resina Kooliner foram realizados espécimes experimentais com 1%, 2,5%, 5% e 7,5% de CHX, para a resina Ufi Gel Hard 1%, 2,5%, 5%, 7,5% e 10% de CHX e para a resina Probase Cold 1%, 2,5% e 5%. Cada grupo era composto por oito espécimes, tendo sido realizados um total de cento e dezoito espécimes.

Todos os espécimes foram sujeitos a um processo de termociclagem que os submeteu a 1000 ciclos de banhos térmicos de 5ºC e 55ºC, o que equivale a um envelhecimento térmico na cavidade oral de um mês. Seguidamente, foi levado a cabo um teste de resistência à flexão de três pontos.

A análise estatística envolveu uma análise descritiva dos valores de resistência à flexão, tendo sido determinados os valores de média, desvio padrão, mediana e amplitude interquartil.

Após análise com recurso ao *software* SPSS Statistics (SPSS Inc., Chicago, IL, USA), verificou-se que os valores não apresentavam uma distribuição normal para as variáveis em estudo (através de testes Shapiro-Wilk) e, por essa razão, os resultados foram submetidos a testes não-paramétricos de acordo com o método de Kruskal-Wallis, seguindo-se comparações múltiplas utilizando testes Mann-Whitney, com correcções de Bonferroni, para determinar se existiam diferenças específicas significativas entre grupos de concentrações de CHX. Um valor de significância de 5% foi a referência em todos os testes estatísticos realizados.

Os resultados da influência da CHX nos valores de resistência à flexão das resinas acrílicas foram divergentes. Para as resinas Kooliner e Ufi Gel Hard não se verificaram diferenças estatisticamente significativas para os diferentes grupos de CHX ($p > 0,05$). Por sua vez, os espécimes do grupo de CHX 5% da resina Probase Cold demonstraram um valor de resistência à flexão inferior comparativamente ao grupo controlo ($p = 0,033$), sendo que as restantes comparações entre grupos não mostraram diferenças estatisticamente significativas ($p > 0,05$).

Uma possível explicação para os resultados similares obtidos pelas duas resinas acrílicas diretas, Kooliner e Ufi Gel Hard, pode encontrar-se nas suas semelhanças químicas. No entanto, as diferenças estruturais e químicas da resina acrílica indireta Probase Cold, comparativamente às restantes, e o seu método de polimerização diferente, em condições de alta temperatura e pressão, podem explicar a razão pela qual a CHX influenciou de modo diferente o grupo 5% CHX desta resina. No grupo 5% CHX do Probase Cold, a presença de partículas de CHX pode ter provocado distúrbios nas cadeias poliméricas da resina acrílica, já que a incorporação de fármacos aumenta a distância intermolecular entre os monómeros das cadeias, registando-se ainda, um aumento de monómero residual o que, consequentemente acarreta com uma menor resistência à flexão.

Os resultados obtidos vão de encontro com os de outros autores que, de modo semelhante, estudaram a incorporação de CHX nas três resinas de rebasamento. A proporção de 10% de CHX na resina Ufi Gel Hard não se revelou nociva para a resistência à flexão contrariamente à conclusão previamente obtida por outro autor embora este tenha utilizado um procedimento de envelhecimento térmico equivalente a três meses.

Concluindo, após envelhecimento térmico equivalente a um mês, as proporções de CHX 1%, 2,5%, 5% e 7,5% na resina Kooliner e as proporções de CHX 1%, 2,5%, 5%, 7,5% e 10% na resina Ufi Gel Hard não reveleram alterar as propriedades de resistência à flexão dos materiais. No entanto, a proporção de CHX 5% na resina Probase Cold mostrou ter uma resistência à flexão diminuída quando comparada com o grupo controlo. Por essa razão, as proporções de CHX que não modificam os valores de resistência à flexão da resina Probase Cold são 1% e 2,5%.

Os resultados obtidos neste estudo são, no entanto, limitados sendo que são necessários mais estudos que investiguem outras propriedades, quer mecânicas quer físicas das resinas acrílicas incorporadas com CHX. É, também, importante desenvolver estudos que realizem protocolos de envelhecimento, não só térmico, mas também químico das resinas. Por fim, será essencial que se concretizem mais estudos de microbiologia e biocompatibilidade.

Palavras-chave: estomatite protética; resinas acrílicas de rebasamento; resistência à flexão; incorporação de fármacos; clorexidina.

Abstract

Objectives: The aim of this study was to evaluate the influence of the concentration of chlorhexidine (CHX) on the flexural strength of three reline acrylic resins, Kooliner, Ufi Gel Hard and Probase Cold, after undergoing a thermal ageing process.

Methods: Distinct proportions of CHX were selected for loading each reline resin. Kooliner had four groups (1%, 2.5%, 5%, 7.5% CHX), Ufi Gel Hard had five groups (1%, 2.5%, 5%, 7.5%, 10% CHX) and Probase Cold had three groups (1%, 2.5%, 5% CHX). Every material had also a control group (0% CHX). Specimens with 64x10x3.3mm were submitted to a thermocycling procedure consisting of 1000 cycles of thermal fluctuations between 5ºC and 55ºC. Afterwards they were tested for flexural strength values using a three point bending device. Results were submitted to nonparametric tests according to the Kruskal-Wallis method, followed by multiple comparisons using Mann-Whitney tests with Bonferroni corrections, being considered the 5% level of significance (α=0.05).

Results: Regarding Kooliner and Ufi Gel Hard specimens, there were no statistical significant differences on flexural strength values among different CHX groups (p>0.05). Results for Probase Cold specimens showed that 5% CHX group had a lower flexural strength value compared to the control group (p= 0.033).

Conclusions: The proportions of 1%, 2.5%, 5% and 7.5% CHX for Kooliner, 1%, 2.5%, 5%, 7.5% and 10% CHX for Ufi Gel Hard and 1% and 2.5% CHX for Probase Cold do not affect the flexural strength values of the acrylic resins after a thermal ageing process equivalent to one month in the oral cavity.

Keywords: denture stomatitis; acrylic reline resins; flexural strength; drug loading; chlorhexidine.

1. Introduction

Residual ridge resorption, after tooth loss, is a chronic and progressive phenomenon of bone remodeling which causes, in individuals wearing removable prosthesis, a diminished denture stability, loss of retention and reduced comfort (Atwood and Coy 1971; Budtz-Jorgensen 1999).

To overcome this physiological phenomena a reline procedure is used since it will enhance the fit of pre-existing denture to the hard and soft tissues (Chow *et al.* 1999; Chopde *et al.* 2012). The materials of choice are usually auto-polymerizing acrylic reline resins, either direct acrylic resins that are cured at the chairside in the dental clinic or indirect acrylic resins which are cured at the laboratory (Neppelenbroek *et al.* 2003; Neves 2012). Auto-polymerizing acrylics turn the reline procedure into a relatively simple, useful and inexpensive treatment (Rawls 2003).

If left untreated a poor fit denture can cause mucosal trauma in denture wearers. This trauma associated with other factors such as the increased age of the user and the increased age of the dentures, a poor denture and oral hygiene, dietary factors, xerostomia, absence of overnight removal, a chronic disease or a compromised immune system can lead to a denture induced stomatitis (Chow *et al.* 1999; Koray *et al.* 2005; Lyon *et al.* 2006; Urban *et al.* 2006; Vanden *et al.* 2008; Gendreau *et al.* 2010; Chopde *et al.* 2012).

The main pathogen related to denture stomatitis is *Candida albicans* due to its ability to adhere and proliferate through tissues of the oral cavity and acrylic resins, and to produce a complex and heterogeneous bacterial biofilm (Waters *et al.* 1997; Akpan *et al.* 2002; Salerno *et al.* 2011).

Candida-associated denture stomatitis is the most frequent form of oral candidiasis and has a preferential localization on the palatal mucosa. Its clinical appearance can be a discrete area of pinpoint inflammation related to de ducts of the palatal mucous glands or an intense erythematous area of the mucosa covered by the denture (Scully *et al.* 1994; Chow *et al.* 1999; Salerno *et al.* 2011). Even though this oral disease is usually asymptomatic it should be treated as it may conduct to more extensive infections and bone resorption. (Wilson *et al.* 1998; Salerno *et al.* 2011).

Treatment of this disorder is usually based on cleansing, relining or even replacement of the denture together with the prescription of antifungal drugs (Cross *et al.* 2004; Neppelenbroek *et al.* 2008; Gendreau *et al.* 2010).

Applying an antifungal agent topically can be highly inefficient due to the rapid drug clearance from the site of infection. Moreover, it is very difficult to obtain a rigid patient compliance and, when drugs are given systemically, only a small concentration of the drug tends to reach the target location and there is a risk of undesired side effects (Chow *et al.* 1999; Amin *et al.* 2009; Salim *et al.* 2012a; Salim *et al.* 2013a). Besides that, even when hygiene solutions are used for denture cleansing, *Candida* strains tend to subsist (Bissel *et al.* 1992; Nikawa *et al.* 2003; Cross *et al.* 2004; Boscato *et al*. 2009).

The feasibility of introducing antimicrobial and antifungal agents in acrylic resins or soft liners acting as drug carriers for the treatment of denture induced stomatitis has been investigated by several researchers (Bueno *et al.* 2013; Salim *et al.* 2013a,b*;* Bertolini *et al.* 2014; Garner *et al.* 2015; Malakhov *et al.* 2016; Wen *et al.* 2016; Sánchez-aliaga *et al.* 2016; Neppelenbroek *et al.* 2018). These drug delivery systems have advantages such as continuous drug release at the infection site maintaining therapeutic levels, minimal risk of systemic toxicity, absence of patient compliance and, when incorporated in reline materials, simultaneous treatment of ill-fitting dentures and *Candida*-related infection (Chow *et al.* 1999; Amin *et al.* 2009; Salim *et al.* 2013).

However, despite the advantages of loading drugs into acrylic resins there can be some influence on the mechanical and physical properties of the material (Schneid 1992; Urban *et al.* 2006; Cunha *et al.* 2008; Casemiro *et al.* 2008*;* Urban *et al.* 2009; Regis *et al.* 2010*;* Paleari *et al.* 2010; Salim *et al.* 2012; Acosta-Torres *et al.* 2012; Rodriguez *et al.* 2012; Sánchez-aliaga *et al.* 2016).

Chlorhexidine is a widely used antiseptic drug with remarkable antifungal, antibacterial and anti-biofilm abilities and a high substantivity (Salim *et al.* 2012; Bueno *et al.* 2013). It has the capability to suppress the adherence of *Candida albicans* to cells or acrylic surfaces and for this reason can inhibit *Candida*-related infections (MacNeill *et al.* 1997*;* Suci *et al.* 2002; Pusareti *et al*. 2009; da Silva *et al.* 2011; Bertolini *et al.* 2014; Iqbal *et al.* 2016; Shino *et al.* 2016).

When loaded into acrylic resins, CHX has shown higher effectiveness in microbiological tests compared to other agents such as fluconazole and nystatin (Amin *et al.* 2009; Ryalat *et al.* 2011; Salim *et al.* 2013a, b). Also, releasing rates with CHX loaded acrylic resins showed interesting results since demonstrated a pattern of higher CHX release at the first two to seven days, that decrease and became steadier for a period of time that ended after twenty eight days (Wilson and Wilson 1993*;* Cao *et al*. 2010; Ryalat *et al*. 2011; Salim *et al.* 2012, 2013*;* Marcelino 2015).

Many microbiological studies found that the concentration of 10% of CHX is probably the most effective against *Candida albicans* (Amin *et al.* 2009; Ryalat *et al.* 2011; Salim *et al.* 2012a, 2013b). However, preliminary results from Costa (2017) established that the minimal concentration of CHX (w/w) would be 2.5% for the reline acrylic resin Kooliner and 5% for Ufi Gel Hard and Probase Cold, since those proportions reveal proper antifungal activity against *Candida albicans*.

The flexural strength of a material is a mechanical property that can be described as the strength needed to fracture an object that is under forces applied in between its end-points, which are fixed. The flexural properties of a removable prosthesis are tested when mastication or impact forces are applied (Anusavice 2003; Paleari *et al.* 2010).

Sousa (2014) found that 10% CHX negatively influenced flexural strength of Kooliner and Probase Cold and Martins (2015) showed that 7.5% CHX group had lower flexural strength values than the control.

Previous studies of loading CHX into acrylic reline resins (Sousa 2014; Martins 2015) were promising because established concentrations of CHX that had antifungal activity with no influence on the properties of the resins. However, these studies point to a short period after polymerization occurred. Oral biomaterials in function are submitted to biodegradation processes that can change their physical and biomechanical properties which lead to the importance of mimic the conditions of the oral cavity through ageing processes.

2. Objectives

The objective of this study was to evaluate the influence of the concentration of CHX on the flexural strength of three different reline acrylic resins, after undergoing a thermal ageing process, according to the following hypotheses:

$H0_1$: Loading Kooliner with different concentrations of CHX does not affect the flexural strength values of the reline acrylic resin.

$H1_1$: Loading Kooliner with different concentrations of CHX affects the flexural strength values of the reline acrylic resin.

$H0_2$: Loading Ufi Gel Hard with different concentrations of CHX does not affect the flexural strength values of the reline acrylic resin.

$H1_2$: Loading Ufi Gel Hard with different concentrations of CHX affects the flexural strength values of the reline acrylic resin.

$H0_3$: Loading Probase Cold with different concentrations of CHX does not affect the flexural strength values of the reline acrylic resin.

$H1_3$: Loading Probase Cold with different concentrations of CHX affects the flexural strength values of the reline acrylic resin.

3. Materials and methods

In the present study three auto-polymerizing acrylic resins were used (Table 3.1), presented in the powder-liquid form.

Two of them are direct reline resins, Kooliner (GC America Inc, Alsip, Illinois, USA) and Ufi Gel Hard (Voco GmbH, Cuxhaven, Germany) (Figure 3.1 a,b) composed by pre-polymerized poly(ethyl methacrylate) particles and the third is an indirect reline resin, Probase Cold (Ivoclar Vivadent AG, Liechenstein) (Figure 3.1 c), formed by pre-polymerized polymethylmetacrylate particles (Arima *et al.* 1995).

Table 3.1-Materials under evaluation in the study.

Product	Manufacturer	Batch number	P/L ratio (g/mL)	Composition	Curing cycle
Kooliner	GC America Inc., Alsip, Illinois, USA	1007201(P) 1008101(L)	1.4/1	P: PEMA L: IBMA	10 minutes 37ºC
Ufi Gel Hard	Voco GmbH, Cuxhaven, Germany	1128441(P) 1134070(L)	1.77/1	P: PEMA L: HDMA	7 minutes 37ºC
Probase Cold	Ivoclar Vivadent AG, Liechtenstein	L49853(P) L43809(L)	1.5/1	P: PMMA L: MMA	15 minutes 40ºC 2-4 bar

P- Powder, L - Liquid, PEMA - polyethyl methacrylate, IBMA – isobutyl methacrylate, HDMA – 1,6 - hexanediol dimethacrylate, PMMA - polymethyl methacrylate, MMA - methyl methacrylate.

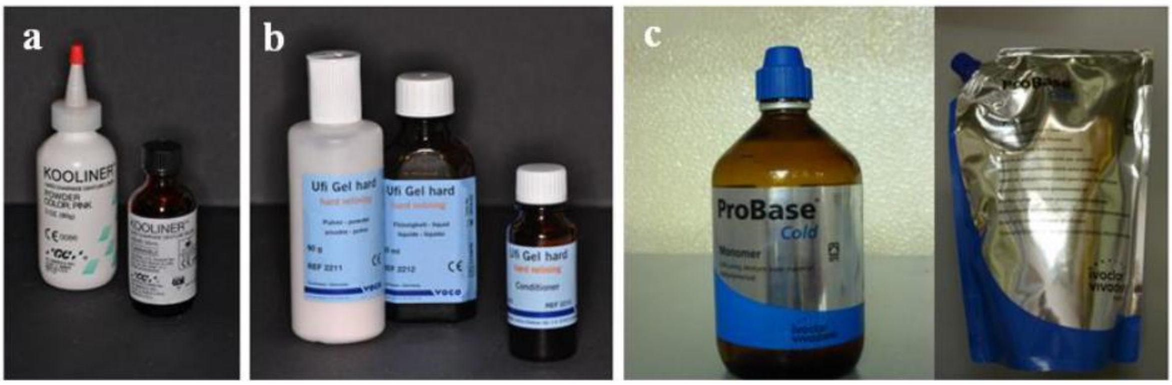

Figure 3.1 - Materials under evaluation in the study; a) Kooliner; b) Ufi Gel Hard; c) Probase Cold.

Preparation of the specimens

The acrylic resins were manipulated according to the manufacturer's instructions (Table 3.1). The liquid was measured using a pipette and the powder was weighed using a precision scale (Mettler Toledo). Afterwards all specimens were loaded with chlorhexidine diacetate monohydrate (Panreac Applichem, Darmstadt, Germany) using a mortar and pestle for homogenization (Figure 3.2), according to the proportions previously established:

- Kooliner : 0%, 1%, 2.5%, 5% and 7.5% of the acrylic resins' powder weight (w/w);
- Ufi Gel Hard: 0%, 1%, 2.5%, 5%, 7.5% and 10% of the acrylic resins' powder weight (w/w);
- Probase Cold: 0%, 1%, 2.5 % and 5% of the acrylic resins' powder weight (w/w).

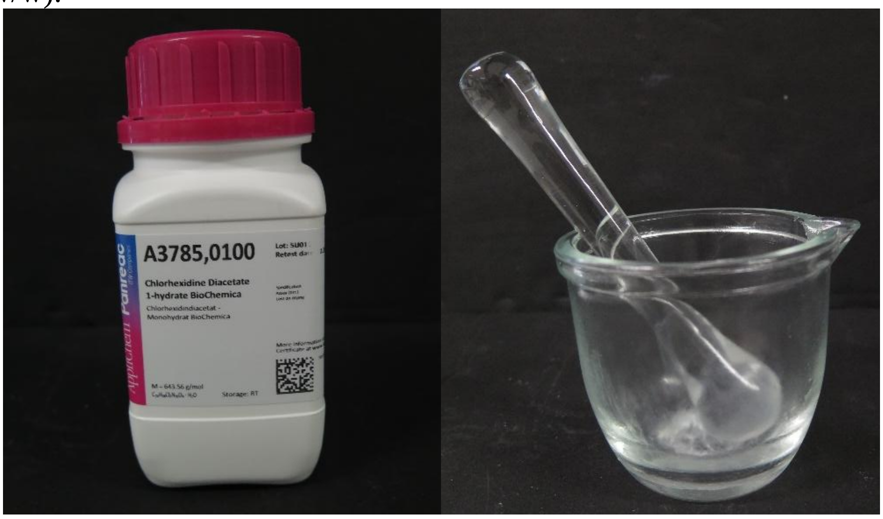

Figure 3.2 – Chlorhexidine diacetate monohydrate; a) Package; b) Incorporation and homogenization.

Eight specimens of each group (*n*=8) were prepared, making a total of one hundred eighteen specimens. Control was represented as the 0% CHX group (Table 3.2).

The specimens of each material were prepared from rectangular shaped stainless steel molds as ISO 20795-1 recommends (ISO 20795-1: 2013), having each specimen approximately 64×10×3.3 mm (Figure 3.3).

For the preparation of each specimen, the stainless steel mold was placed on a glass plate covered by a polyester sheet. The materials were prepared and placed into the mold. A new polyester sheet and glass plate were positioned on top of the mold (Figure 3.3).

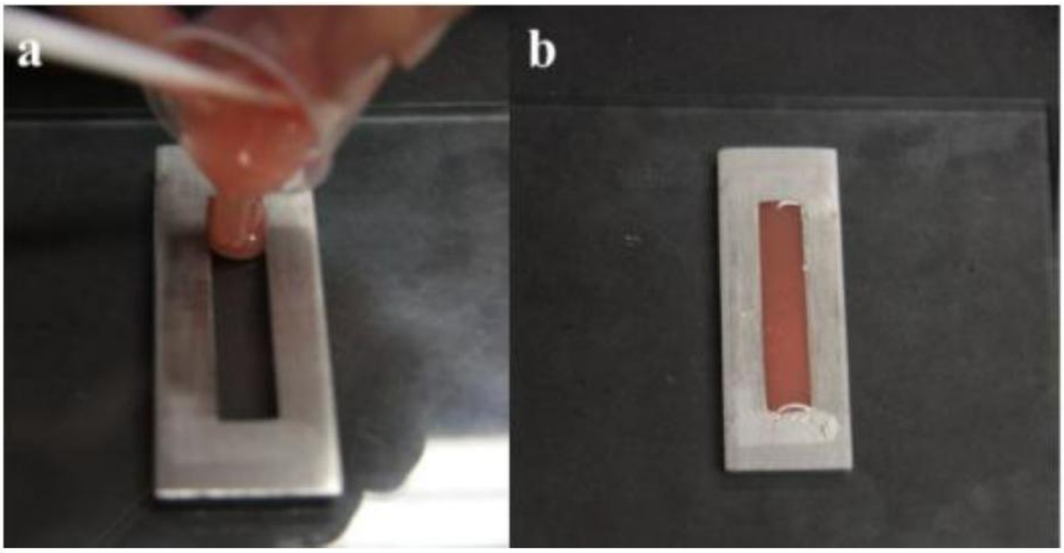

Figure 3.3 – Preparation of the specimens; a) Homogeneous mixture of liquid and powder formulations is placed in the stainless steel mold; b) Pre-polymerized specimen and mold between polyester sheets and glass plates.

uring the polymerization of the direct reline resins, the specimens were kept under compression at 37±2ºC for the time stablished by the manufacturer (Table 3.1), in order to simulate the intraoral curing cycle of the materials. On the other hand, polymerization of the indirect reline resin was carried out in a pressure device (Ivomat, Ivoclar Vivadent, Liechenstein) (Figure 3.4) at recommended time, temperature and pressure (Table 3.1).

Figure 3.4 – Ivomat pressure device.

After polymerization, the samples were removed from the molds and the edges of each sample were polished with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL), on a polisher with constant refrigeration (Figure

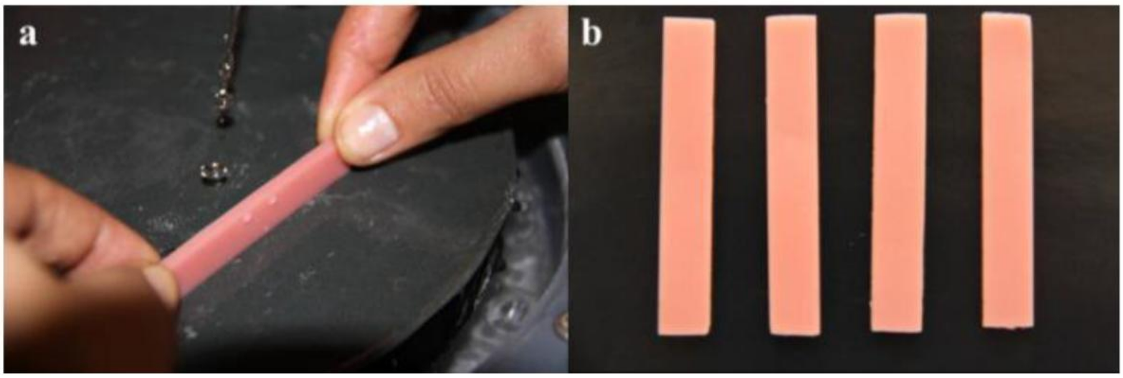

Figure 3.5 - Preparation of the specimens. After polymerization and removal of the specimen from the molds; a) Irregularities were removed; b) Examples of polymerized Kooliner specimens.

3.5).

All specimens were submitted to thermal ageing process, being exposed to 1000 cycles of thermal fluctuations between 5ºC and 55ºC (20 seconds each bath), with 5 seconds of dwell time, in a specific machine (Refri 200-E, Aralab, Cascais, Portugal) (Figure 3.6).

Figure 3.6 – Thermocycling machine Refri 200-E, Aralab

Flexural Strength Test

A servo-hydraulic universal testing machine (Instron Model 4502) was used to perform flexural strength tests using a three point bending device (Figure 3.7). Firstly, the width and thickness were measured in each specimen with a digital micrometer of 0.01mm precision (Mitutoyo Digimatic, MFG. Co., Ltd Tokyo, Japan). Then their averages were introduced in the software just before testing.

A crosshead speed of 5mm per minute was selected and the distance between supports was 50mm, as described elsewhere (ISO 20795-1: 2013).

Load was applied until failure and the fracture load was recorded in Newtons (N). The flexural strength was expressed in megapascal (MPa) and calculated using the formula:

$$FS = \frac{3Wl}{2bd^2}$$

Where *FS* is the flexural strength, *W* is the maximum load before fracture (N), *l* is the distance between supports (50mm), *b* is the specimen's width (mm) and *d* is the specimen's thickness (mm).

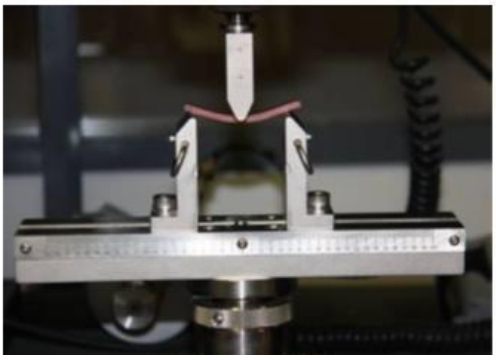

Figure 3.7 - Specimen submitted to 3 point loading flexural strength test in a universal machine.

Statistical Analysis

Descriptive statistics of flexural strength values were carried out being determined the mean, median, standard deviation and interquartile range per group.

Since data did not follow a normal distribution for the studied variables (verified by Shapiro-Wilk normality tests), the results were submitted to the nonparametric tests according to the Kruskal-Wallis method, followed by multiple comparisons using Mann-Whitney tests with Bonferroni corrections to determine whether there were specific significant differences among materials and groups.

In all statistical tests, it was considered the 5% level of significance (α=0.05).

Data were statistically analyzed using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA).

4. Results

The descriptive analysis is summarized on Table 4.1. including mean, standard deviation, median and interquartile range values of flexural strength per each group.

Table 4.1- Descriptive analysis for each material.

Material	**CHX concentration**	**Flexural strength (MPa)**	
		M (SD)	**Mdn (IQR)**
Kooliner	0%	81.3 (14.19)	78.9 (26.00)
	1%	92.9 (17.90)	93.8 (35.86)
	2.5%	92.9 (8.51)	91.1 (17.63)
	5%	94.9 (13.70)	92.5 (13.27)
	7.5%	87.2 (8.91)	86.9 (17.94)
Ufi Gel Hard	0%	66.7 (10.97)	67.0 (20.10)
	1%	68.1 (13.99)	67.3 (25.70)
	2.5%	77.7 (9.15)	76.1 (13.83)
	5%	74.2 (9.38)	75.6 (13.60)
	7.5%	75.4 (7.27)	77.3 (11.92)
	10%	66.3 (7.01)	67.7 (12.56)
Probase Cold	0%	163.6 (39.48)	180.0 (57.31)
	1%	148.4 (19.01)	153.7 (11.20)
	2.5%	142.4 (20.13)	146.3 (17.76)
	5%	124.9 (18.15)	124.6 (38.75)

M= mean; SD= standard deviation; Mdn= median; IQR= Interquartile range; n=8 for each group.

Kooliner specimens (Figure 4.1.) showed no differences of flexural strength among groups of different CHX concentrations loaded in the resin (p>0.05).

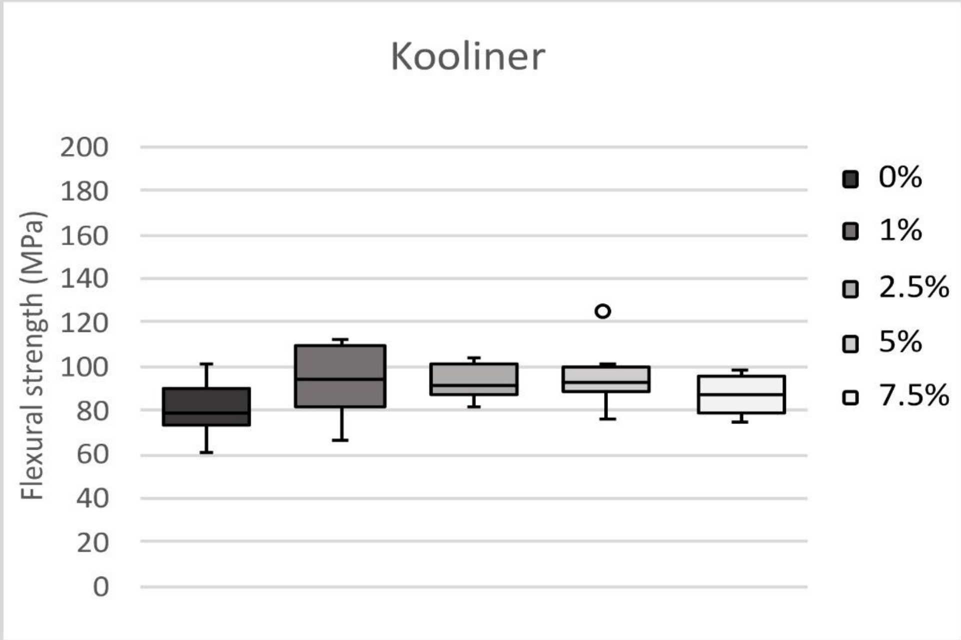

Figure 4.1. – Mean, standard deviation, median and interquartile range of flexural strength values (MPa) for different groups of CHX of Kooliner.

Also, groups of Ufi Gel Hard (Figure 4.2.) showed no statistical significant differences on flexural strength values ($p>0.05$).

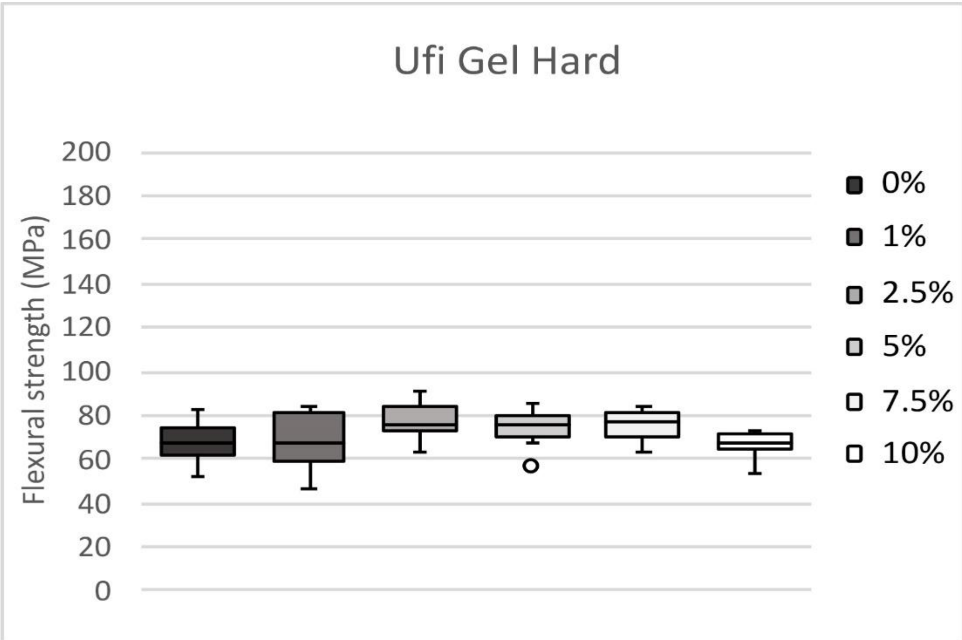

Figure 4.2. – Mean, standard deviation, median and interquartile range of flexural strength values (MPa) for different groups of CHX of Ufi Gel Hard.

Considering Probase Cold (Figure 4.3.), 5% CHX group had lower flexural strength values compared to the 0% CHX (control) group (*p*= 0.033). The remaining comparisons between groups did not evidence statistical significant differences on flexural strength values (*p*>0.05).

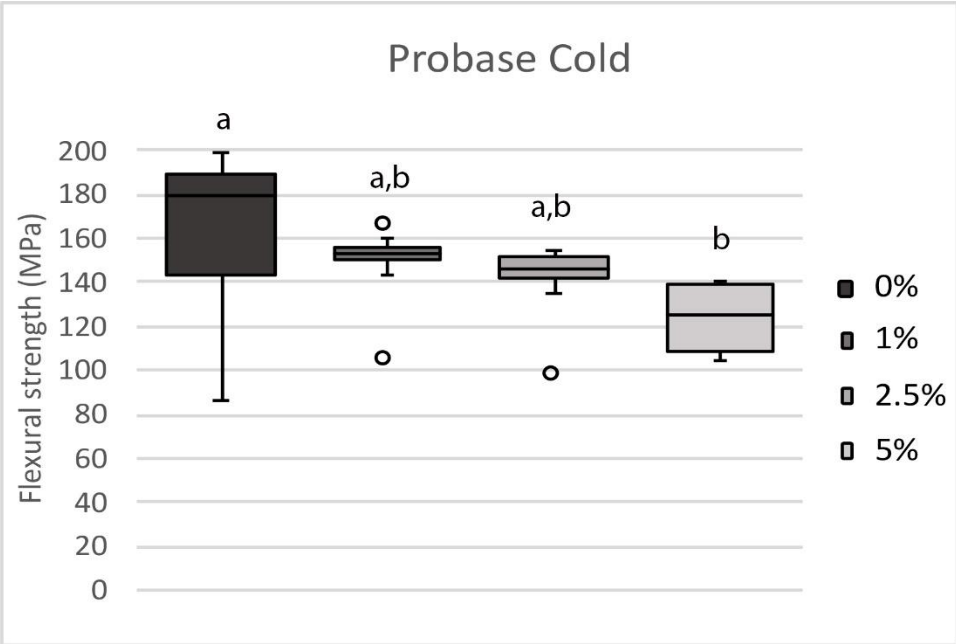

Figure 4.3. – Mean, standard deviation, median and interquartile range of flexural strength values (MPa) for different groups of CHX of Probase Cold. Groups assigned with the same letter (a/b) show no significant statistical differences between them.

5. Discussion

The purpose of this study was to verify if there was an influence on the flexural strength of three types of reline resins loaded with various concentrations of CHX. Flexural strength was the strength property selected since it is one of the most significant features when assessing dental polymers and it's a helpful property for predicting the clinical performance of the denture when subjected to mastication forces (Anusavice 2003; Casemiro *et al.* 2008; Al-Haddad *et al.* 2014).

The three reline resins were chosen for their differences in chemical composition and structural arrangement. Kooliner has the monomer isobutylmethacrylate and forms a simple non-crosslinking net when polymerization is complete. Ufi Gel Hard has the monomer 1,6-hexanodioldimethacrylate and forms a more complex crosslinking net. Probase Cold, a resin that polymerizes on the laboratoty, has methylmethacrylate as the monomer, forming a net with a reduced percentage of uncured monomer (Arima *et al*. 1995; Arima *et al.* 1996).

Other objective of the present study was to submit the specimens to a thermocycling ageing procedure. This was done in order to simulate the temperature fluctuations occurring in the oral cavity whenever there is ingestion of food and drinks at different temperatures (Palmer *et al*. 1992). It is important to evaluate the impact of thermal ageing on the acrylic resins because it is known that these variations of temperature can produce mechanical stresses and, consequentially, induce crack propagation and diminish the mechanical properties of acrylic based materials (Neppelenbroek *et al.* 2006; Neves 2012). In the present study, the thermocycling method consisted of 1000 cycles of thermal fluctuations between 5ºC and 55ºC, which corresponds to 6 weeks of intraoral temperature variation (Gale and Darvell. 1999). This particular period of time was selected due to the fact that previous studies, focused on the releasing rates of CHX loaded in acrylic resins (Wilson and Wilson 1993*;* Cao *et al*. 2010; Ryalat *et al*. 2011), showed that most of the CHX incorporated is released from the carrier devices in the first twenty eight days, approximately one month, and after this time the antifungal dentures become gradually less efficient, which implies that reline procedures with reline resins loaded with CHX shouldn't extend longer than this period of time.

The CHX concentrations selected were based on previous mechanical studies (Sousa 2014; Martins 2015), focused on 0%,1%, 2.5%,5%, 7.5% and 10% groups of CHX loaded on the same three acrylic reline resins of the present study.

Sousa (2014) referred that loading 10% CHX could negatively influence flexural strength of Kooliner and Probase Cold, so they were excluded for the present study. However, the proportion of Ufi Gel Hard 10% CHX was included in the study because, according to the study from Sousa (2014), the specimens of Ufi Gel Hard 10% CHX didn't evidence lower flexural strength values when compared to the control group (0% CHX). On the other hand, Martins (2015) found a negative impact on the flexural strength of Probase Cold when 7.5% CHX was loaded on the resin specimens. So, it was settled in the present study to include groups of Probase Cold only till 5% CHX.

In the present study, the influence of different CHX concentrations on the flexural strength diverged between reline resins. Concerning Kooliner and Ufi Gel Hard groups there were no statistical significant differences between specimens with different CHX loading. The fact that both Ufi Gel Hard and Kooliner groups weren't affected by the CHX loading might be explained by their similar chemical constitution, being both composed of pre-polymerized poly(ethyl methacrylate) particles (Arima *et al*. 1995).

The results are similar to those obtained by Martins (2015) because specimens of Kooliner and Ufi Gel Hard incorporated with CHX in the concentration of 1%, 2.5%, 5% and 7.5%, even after being subjected to thermal ageing, didn't evidence a negative impact on their flexural strength. On the other hand, results for Ufi Gel Hard 10% CHX do not corroborate those found by Sousa (2014) since the author concluded that after thermal ageing equivalent to three months, groups of Ufi Gel Hard with 10% CHX had a reduction of flexural strength. Therefore, it can be stipulated that doing a reline procedure with Ufi Gel incorporated with 10% CHX for a period of time longer than one month can influence negatively its flexural strength.

At this time it may be concluded that the first and second hypothesis cannot be rejected since no statistical significant differences between specimens with different CHX loading were found.

The third null hypothesis may, however, be rejected since Probase Cold 5% CHX group had significant lower flexural strength compared to the control. A diminished flexural strength can result in a greater incidence of fracture when the acrylic is subjected to occlusal stress (Cunha *et al.* 2008). Even so, in this particular situation, the 5% CHX group still had a flexural strength that was clinical accepted by the ISO 1567 standard (65 MPa) (Paleari *et al.* 2010; Acosta-Torres *et al.* 2012).

Probase Cold was the only resin that demonstrated significant variances between groups with different CHX concentrations. This acrylic resin has a different chemical composition, since it is composed of pre-polymerized polymethylmetacrylate particles, and has different structural arrangement compared to the other resins studied. Moreover, unlike Kooliner and Ufi Gel Hard, its curing cycle is accomplished under high temperature and pressure. These reasons might explain the negative influence of CHX on Probase Cold specimens' flexural strength. The result obtained for Probase Cold 5% CHX group is in accordance with many other studies (Addy *et al.* 1981; Casemiro *et al.* 2008; Cunha *et al.* 2008; Regis *et al.* 2010; Paleari *et al.* 2010; Acosta-Torres *et al.* 2012; Rodriguez *et al.* 2012; Sousa 2014; Martins 2015) that revealed a reduction on flexural strength values of resins after being combined with antimicrobial agents, although only Sousa (2014) implemented a thermal ageing procedure. Some authors have also described an inverse proportional relation between the concentration of the antimicrobial introduced and the flexural strength values, meaning that higher amounts of drug loaded in the acrylic resins are translated in lower flexural strength values of the materials (Addy *et al.* 1981; Regis *et al.* 2010; Paleari *et al.* 2010).

According to Addy (1981), the physical presence of the CHX particles in the resin matrix may disturb the physical form of polymers (Addy *et al.* 1981). In fact, Rodrigues (2012) explained the decrease of flexural strength of the denture base acrylic resin by the possible increase of intermolecular distance between monomers of the polymer chains after the incorporation of the antimicrobial monomer 2-Tert-Butylaminoethyl Methacrylate (Rodriguez *et al.* 2012). Paleari (2010) associated the diminished flexural strength of the denture base acrylic resin loaded with 2-Tert-Butylaminoethyl methacrylate to the presence of a higher amount of residual monomer and a lower conversion degree of the acrylic resin (Paleari *et al.* 2010).

Therefore, the reduction of the flexural strength of Probase Cold 5% CHX group could be substantiated by the increase of intermolecular distance of monomers in the polymer chains and the increase of residual monomer.

Recent preliminary results from a microbiological study by Costa (2017) established that the most effective concentration of CHX (w/w) against *Candida albicans* would be 2.5% for Kooliner and 5% for both Ufi Gel Hard and Probase Cold.

Considering the results achieved by this study and by Costa (2017), it can be concluded that the proportions of 2.5% CHX for Kooliner and 5% CHX for Ufi Gel Hard are valid because, besides being effective against *Candida albicans,* there is also no influence on the acrylic resins flexural properties. On the other hand, introducing a concentration of 5% CHX in Probase Cold may not be advisable on future investigations because, although microbiologically effective, the present study verified a negative impact on the flexural strength values of this specific group.

With this study it was possible to achieve important conclusions regarding the concentrations of CHX possible to incorporate in reline resins without influencing their flexural strength after these have been submitted to thermal ageing of one month period.

However, the results found are limited being important to investigate other mechanical and physical features of the acrylic resins loaded with CHX after not only thermal but also chemical ageing. It’s also essential to associate more microbiological and biocompatibility tests

6. Conclusions

- The first null hypothesis may be accepted since there were no variations on flexural strength values between all groups of Kooliner;
- The second null hypothesis may, as well, be accepted because there wasn't evidence of alterations on flexural strength values for different Ufi Gel Hard groups.
- The third null hypothesis, concerning Probase Cold groups, may, however, be rejected due to the lower flexural strength values from group 5% CHX compared to control.

Therefore, it can be established that the concentrations of 1%, 2.5%, 5% and 7.5% CHX for Kooliner, 1%, 2.5%, 5%, 7.5% and 10% CHX for Ufi Gel Hard and 1% and 2.5% CHX for Probase Cold do not affect the flexural strength values of the acrylic resins after a thermal ageing equivalent to one month of oral environment.

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Appendices

Appendix 1 – List of tables

Appendix 2 – List of figures

Appendix 3 – List of abbreviations

CHX - Chlorhexidine diacetate monohydrate

FS – Flexural Strength

HDMA – 1,6 - hexanediol dimethacrylate

IBMA – isobutyl methacrylate

ISO – International Organization for Standardization

MMA - methyl methacrylate

MPa - Megapascal

PEMA - polyethyl methacrylate

PMMA - polymethyl methacrylate

Appendix 4 – Flexural Strength values

Material	Proportion	Specimen	Load at Yield [KN]	Width [mm]	Thickness [mm]	Flexural Strength [Mpa]
Probase Cold	0%	1	0.2341	10.19	3.41	148.1766374
		2	0.123	10.13	3.24	86.74948577
		3	0.2627	10.17	3.08	204.220319
		4	0.2517	10.24	3.14	186.9757251
		5	0.276	10.27	3.23	193.1945439
		6	0.2544	10.37	3.18	181.9471989
		7	0.2712	10.3	3.33	178.0841455
		8	0.1867	10.22	3.25	129.714332
	1%	1	0.2022	10.09	3.09	157.4107143
		2	0.2109	10.21	3.15	156.1316659
		3	0.214	10.28	3.19	153.4265629
		4	0.1545	10.23	3.3	104.0126673
		5	0.2	10.14	3.21	143.5632361
		6	0.2145	10.15	3.22	152.8659552
		7	0.2176	10.15	3.11	166.2391594
		8	0.2164	10.17	3.22	153.9167284
	2,5%	1	0.1894	10.26	3.11	143.1439836
		2	0.1249	10.17	3.08	97.09599484
		3	0.2085	10.16	3.23	147.526001
		4	0.1934	10.09	3.09	150.5600007
		5	0.2136	10.09	3.12	163.1030782
		6	0.2244	10.25	3.48	135.5819147
		7	0.2255	10.15	3.26	156.7857426
		8	0.228	10.19	3.4	145.1657266
	5%	1	0.1516	10.35	3.25	104.0048023
		2	0.1592	10.29	3.13	118.4405122
		3	0.1976	10.11	3.15	147.7324637
		4	0.2071	10.3	3.2	147.2665731
		5	0.1557	10.93	3.2	104.3349368
		6	0.1574	10.73	3.16	110.1772946
		7	0.1828	10.26	3.13	136.3959324
		8	0.1937	10.14	3.31	130.766633

Material	Proportion	Specimen	Load at Yield [KN]	Width [mm]	Thickness [mm]	Flexural Strength [Mpa]
Kooliner	0%	1	0.0964	10.34	3.4	60.48670464
		2	0.1223	10.34	3.34	79.51961121
		3	0.1351	10.28	3.35	87.82818008
		4	0.109	10.5	3.38	68.1498747
		5	0.1171	10.19	3.38	75.441545
		6	0.1386	10.18	3.17	101.6150865
		7	0.1331	10.26	3.14	98.68080007
		8	0.124	10.64	3.34	78.35169335
	1%	1	0.0989	9.96	3.21	72.27500856
		2	0.1262	10.16	3.33	84.01144281
		3	0.1272	10.68	3.68	65.96012723
		4	0.1607	10.02	3.28	111.8051301
		5	0.1562	10.31	3.41	97.7180675
		6	0.1618	10.36	3.28	108.8760454
		7	0.1477	10.04	3.13	112.6209978
		8	0.1292	10.21	3.25	89.85273748
	2,5%	1	0.1443	10.18	3.25	100.6498413
		2	0.132	10.55	3.38	82.13898547
		3	0.14	10.5	3.46	83.53102342
		4	0.1552	10.01	3.36	103.0007408
		5	0.145	10.01	3.48	89.70914143
		6	0.1286	10.19	3.2	92.43322191
		7	0.1248	10.26	3.21	88.53569955
		8	0.1612	10.41	3.35	103.4870381
	5%	1	0.1448	10.18	3.28	99.15950722
		2	0.1237	10.01	3.28	86.14879321
		3	0.1557	10.08	3.07	122.9171814
		4	0.1102	10.09	3.28	76.13844525
		5	0.1462	10.13	3.28	100.6123987
		6	0.1283	10.12	3.26	89.46892252
		7	0.1415	10.3	3.3	94.61338896
		8	0.1374	10.09	3.36	90.46451831
	7.5%	1	0.1182	9.92	3.22	86.18969113
		2	0.1166	10.25	3.28	79.30275243
		3	0.1474	10.11	3.34	98.01999086
		4	0.1383	10.11	3.24	97.73323347
		5	0.1171	10.56	3.08	87.67036351
		6	0.1156	10.8	3.19	78.88855041
		7	0.1307	10.32	3.17	94.52324644
		8	0.1143	10.45	3.3	75.32919451

Material	Proportion	Specimen	Load at Yield [KN]	Width [mm]	Thickness [mm]	Flexural Strength [Mpa]
Ufi Gel Hard	0%	1	0.0895	10.19	3.12	67.67074016
		2	0.0915	9.99	3.26	64.63707111
		3	0.0710	10.19	3.18	51.6762735
		4	0.1074	10.05	3.11	82.86644444
		5	0.1003	10.18	3.18	73.0735453
		6	0.0751	10.16	3.28	51.52995996
		7	0.1020	9.94	3.19	75.6299276
		8	0.0913	9.97	3.22	66.24073447
	1%	1	0.0988	10.54	3.53	56.41936402
		2	0.1248	10.11	3.59	71.83495036
		3	0.1093	9.92	3.63	62.71284499
		4	0.1443	10.13	3.62	81.52691486
		5	0.1241	10.03	3.34	83.18388089
		6	0.1394	10.28	3.49	83.49876818
		7	0.0949	10.19	3.44	59.02505585
		8	0.0597	10	3.1	46.59209157
	2,5%	1	0.1431	10.21	3.49	86.30268375
		2	0.1567	10.66	3.77	77.56938617
		3	0.1322	10.26	3.6	74.56591582
		4	0.1496	10.1	3.49	91.20541614
		5	0.1129	10.41	3.6	62.76239015
		6	0.1031	10.21	3.26	71.26216074
		7	0.1223	10.07	3.29	84.15238984
		8	0.1078	9.92	3.32	73.94216879
	5%	1	0.1148	10.26	3.53	67.34515858
		2	0.1397	10.08	3.48	85.82990849
		3	0.1047	10.25	3.2	74.81421494
		4	0.1404	10.27	3.51	83.22306278
		5	0.0941	10.13	3.52	56.22845034
		6	0.1122	10.08	3.27	78.07249938
		7	0.1097	9.94	3.29	76.4697571
		8	0.1019	9.96	3.28	71.3228061

Ufi Gel Hard	7.5%	1	0.1217	10.23	3.31	81.43671005
		2	0.1359	9.93	3.61	78.76206024
		3	0.1126	9.99	3.34	75.7776673
		4	0.1105	10.12	3.6	63.18849729
		5	0.1119	10.14	3.47	68.73761279
		6	0.0999	10.02	3.25	70.79332459
		7	0.1115	9.85	3.25	80.37725648
		8	0.1154	10.26	3.16	84.478374
	10%	1	0.1163	10.08	3.59	67.14157874
		2	0.0916	10.5	3.37	57.61129483
		3	0.1050	10.09	3.38	68.31656092
		4	0.1073	10.26	3.32	71.16024869
		5	0.0805	10.39	3.28	54.01245392
		6	0.0933	10.11	3.23	66.34171693
		7	0.1024	10.18	3.21	73.2155581
		8	0.0957	10.07	3.13	72.75369507